IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellants:	Lara Madison, Gjalt W. Huisman and Oliver P. Peoples		
Serial No.:	09/235,875	Art Unit:	1638
Filed:	January 22, 1999	Examiner:	Russell Kallis

For: *TRANSGENIC SYSTEMS FOR THE MANUFACTURE OF POLY(3-HYDROXY-BUTYRATE-CO-3-HYDROXYHEXANOATE)*

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Commissioner for Patents
P.O. Box 1450
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REPLY BRIEF

Sir:

This is a reply brief to the Examiner's Answer mailed April 9, 2007, and the Examiner's Answer mailed on June 14, 2007, in the above-referenced application. A Request for Oral Hearing was submitted with the Reply Brief filed on June 11, 2007, in response to Examiner's Answer mailed on April 9, 2007. The Commissioner was authorized to charge $1000, the fee for a Request for Oral Hearing for a large entity, to Deposit Account No. 50-3129.

It is believed that no fee is required with this submission. However, should a fee be required, the Commissioner is hereby authorized to charge the fee to Deposit Account No. 50-3129.

3) STATUS OF CLAIMS ON APPEAL

Claims 1, 6, 7, 10, 14, 16-21 and 35-39 are pending and on appeal. Claims 2-5, 32 and 33 were cancelled in the amendment and response filed on July 29, 2002. Claims 11-13, 22-27 and 31 were cancelled in the amendment and response filed on March 10, 2003. Claims 8 and 9, 29-30 and 34 were cancelled in the amendment and response filed on February 3, 2004. New claims 35-39 were added in the amendment and response filed on June 1, 2006.

(6) REVISED GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

It is not clear what rejections are withdrawn and what new rejections have been made, in the paragraph at the bottom of page 2 and page 3 of the Examiner's Answer. The following is believed to be correct.

i) Claims 24-26 were cancelled. Claims 37-39 satisfy the written description requirement under 35 U.S.C. §112, first paragraph.

ii) claim 39 is definite as required by 35 U.S.C. §112 second paragraph.

iii) claims 1, 7, 10, 14, 16, 18-21, 36 and 38 are novel as required by 35 U.S.C. §102(b) over Fukui, et al., *J. Bacteriol.*, 179:4821-4830 (1997) ("Fukui")

iv) claims 1, 6-7, 10, 14, 16-21 and 35-39 are non-obvious as required by 35 U.S.C. §103(a) over Fukui in view of U.S. Patent No. 5,470,727 to Mascarenhas, et al. ("Mascarenhas") and further in view of Schubert, et al., *J. Bacteriol.* 170(12):5837-5847 (1988) ("Schubert"), Boynton, et al., J. Bacteriol., 178(11):3015-3024 (1996) ("Boynton"), U.S. Patent No. 5,512,482 to Voelker, et al. ("Voelker") and unspecified portions of Appellants' specification.

(7) ARGUMENTS

(b) Rejection Under 35 U.S.C. § 112 first paragraph-written description

Analysis

Claims 24-26 have been cancelled. Claims 37-39 satisfy the written description requirement. This is a **new ground of rejection.**

Claim 37 depends from claim 18 and further defines the one or more fatty acid biosynthetic enzymes as being from *Nocardia salmonicolor*. It is well established that one does not need to provide in the patent applicationall information known to those of ordinary skill in the art in order to comply with the written description requirement. Referring to Example 1, it is clear that one of ordinary skill in the art can obtain these enzymes using the reagents and sequences described therein, as appellants did. It is not necessary to sequence these enzymes in order to use them, as appellants also demonstrate. See also Hall, et al., "Cloning of the Nocardia corallina polyhydroxyalkanoate synthase gene and production of poly-(3-hydroxybutyrate-co-3-hydroxyhexanoate) and poly-(3-hydroxyvalerate-co-3-hydroxyheptanoate)" Can. J. Microbiol. 44,7 (July 1998), cited by the examiner in the office action mailed October 11, 2000. The sequences were also known in other bacteria, such as *E coli*, as demonstrated by the Pramanik, et al., J. Bacteriol. 137(1):469-473 (1979), cited in the information disclosure statement considered on October 6, 2000.

Medline and the specification at least at page 14, lines 19-21 indicates that **the amino acid sequence** encoding the enzyme are known. As disclosed in the specification, genes homologous to the FaoAB complex can be isolated from other source, using techniques known in the art. Similarly with respect to claim 37, fatty acid biosynthetic enzymes are known in the art. Claim 37 requires that the bacteria express one or more fatty acid biosynthetic enzymes

from *N. salmonicolor*. One of ordinary skill in the art knows what fatty acid biosynthetic enzymes are (*See* for Example, U.S. Patent No. 6, 586,658 to Peoples, et al. "Peoples" evidence relied upon in the Examiner's answer), and the specification describes at least one gene from *N. salmonicolor* involved in the fatty acid biosynthetic pathway (see the specification at least at page 12, lines 1-15 and page 15, lines 4-23).

Claim 38 depends from claim 18 and further requires that the one or more fatty acid biosynthetic enzymes epimerizes S-3-hydroxyhexanoyl-CoA to R-3-hydroxyhexanoyl-CoA. Claim 39 requires that the enzyme of claim 38 be from *Pseudomonas putida* FaoAB complex.

As noted above, it is well established that one does not have to describe that which is known and available. Epimerases are known in the art. However, the specification at least from page 14, line 3 until page 15, line 2 discloses sources of epimerases.

Example 6 at page 25 describes the isolation and use of these enzymes. See also Figure 5, identifying the enzymes.

Furthermore, the word "epimerase," and "fatty acid biosynthetic enzymes" (as identified further in Figure 5) classify enzymes by their chemical structure and readily convey distinguishing information concerning identity, *via* structure and function, such that one of ordinary skill in the art could easily visualize the identity of the members of each classification.

One of ordinary skill in the art knows that functional definitions provide structural information commonly possessed by all members of each class. Over 30 years ago, Nobel Laureate Christian B. Anfinsen proved that a protein's "knowledge" of how to fold is stored in its sequence of amino acids. It is this fold that determines the protein's functionality (i.e. substrates recognized, reactions catalyzed, targeted protein binding, etc.). Conversely, a particular function can be directly attributed to particular folds determined by specific, or conserved, sequences of

amino acids. It is well established in the art that structure–function relationships do exist, and it is no more prevalent than within families of proteins, such as those that drive the specific reactions of claim 37. The written description requirement can be met by a functional description of claimed materials, if coupled with a known or disclosed correlation between function and structure.

The Examiner alleges that the claims require an enzyme that epimerizes S-3-hydroxyhexanoyl-CoA to R-hydroxyhexanoyl-CoA form an unspecified source and an unspecified structure and the FaoAB complex from *P. putida* of unspecified identity. It is not clear what the Examiner means by "unspecified identity" in reference to the FaoAB complex from *P. putida.* The specification at least at page 14, lines 21-26 describes epimerase activity for the FaoAB complex from *P. putida*, which as Exemplified by Peoples, has been isolated and characterized (*See* Example 1 of Peoples). Information which is well known in the art need not be described in detail in the specification. See, e.g., *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379-80, 231 USPQ 81, 90 (Fed. Cir. 1986).

The Examiner is clearly overlooking the Board of Patent Appeals and Interferences' and the Federal Court's emphasis that written description is determined from the perspective of what the specification conveys to one skilled in the art citing *In re GPAC Inc.*, 57 F.3d 1573, 1579, 35 USPQ2d 1116, 1121 (Fed. Cir. 1995) and *Vas Cath*, 935 F.2d at 1563-64. As already stated, the enzymes recited in claims 37 and 38 as well as their sources are known in the art and described in the specification. Therefore, claims 37-39 are satisfy the written description requirement.

(c) Rejection Under 35 U.S.C. § 112 second paragraph-indefiniteness

Claim 39 was rejected under 35 U.S.C. § 112 for being indefinite for reciting "the enzymes", instead of "the enzyme".

The requirement under 112 is that the claim define the metes and bounds of the claimed subject matter so that it is understood by one of skill in the art. Anyone of skill in the art would understand that "the enzyme" should be plural to be correct grammatically. Appellants will amend claim 39 correct this typographical error, to recite "the enzyme", thereby providing antecedent basis for claim 39. However, such an amendment is not required for compliance with 112.

(d) Rejections Under 35 U.S.C. § 102

Claims 1, 7, 10, 14, 16, 18-21, 36 and 38 are not anticipated by Fukui.

Fukui

Fukui discloses production of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) by the β-oxidation pathway, by providing substrates of C6 or longer fatty acids, specifically hexanoate and octanoate. This is inherently different since the pathways and enzymes required to arrive at the PHBH are different since the substrates are different. These are not interchangeable.

Enzyme specificity is determined by the substrate. Absent evidence that these organisms express *all the claimed* enzymes required to produce the necessary substrates to produce poly(hydroxybutyrate-co-3hydroxyhexanoate) from butanol or butyrate, AND that Fukui provided to these bacterial butanol or butyrate in an effective amount to produce poly(hydroxybutyrate-co-3hydroxyhexanoate), the prior art does not inherently disclose the claimed method.

Fukui only looks at PHA-negative mutants of *A. eutrophus* and *P. putida*, organisms that normally make PHB. One does not know all of the enzymes that are expressed by these organisms or what defect has interrupted polymer production. Complementation studies are not predictive of one's ability to engineer a pathway into an organism which does not normally make polymer at all, such as *E. coli*. What is known, as discussed below, is that Fukui's bacteria utilize long chain (C6 and C8) substrates, not the short chain butanol or butyrate.

It is clear that the claimed method is very different from that disclosed in Fukui since different pathways and enzymes are utilized. There are many pathways that can ultimately produce PHAs, as described in the specification and Figures 1-5, and different carbon sources that can be utilized by the PHA-producing organism. The method disclosed by Fukui utilizes the pathway shown in the specification in Figure 4 (β oxidation), which is also shown in Fukui (Figure 4, page 4829). The bacteria used to produce the PHAs are grown on fructose, gluconate, hexanoate, or octanoate (all C6 or C8 substrates) as a carbon source (Fukui, page 4823, column 2). This is different from the claimed method which utilizes short carbon chain substrates. The claimed method provides transgenic microorganisms that produce the co-monomer 3-hydroxyhexanoic acid from cheaper shorter carbon chain feedstock such as butyrate or butanol (please see the specification at page 5, lines 6-10). Fukui does not disclose the claimed method. The Examiner asserted that the limitation "wherein the bacteria can utilize butyrate or butanol" has no patentable weight because it points to an inherent property of bacteria and not a feature of the method such as an active method step. The Examiner has provided no basis for arriving at the conclusion that the ability to utilize butyrate or butanol is inherent to bacteria. Indeed, it is contrary to general knowledge, and in particular the field of polyhydroxybutyrate production, since the only PHA originally identified in bacteria was PHB. Bacteria do not naturally produce

copolymers of longer chain PHAs. The original polymerase isolated in 1989 as described in the background of the application at pages 1-2 only utilized short chain substrates. It was only later that a second polymerase was isolated form *P. oleovarans* that could utilize longer chain substrates.

The Examiner cited to Schubert, page 5845 Fig 2 and Doi, et al., *Appl. Microbiol Biotecnool.*, 28:330-334 (1988) for a disclosure of the utilization of butyrate as a carbon source. For Fukui to anticipate the limitation that the bacteria can utilize butyrate or butanol and the bacteria will produce polyhydroxybutyrate-co-3-hydroxyhexanoate, the disclosure has to be present in Fukui. None of Schubert or Doi disclose the use of butanol or butyrate by *A. eutrophus* resulting in the production of a PHA containing 3-hydroxyhexanoate units, only 3HB.

The Examiner also asserted that phbB from *R. eutropha* inherently possesses the ability to convert butyryl-CoA and acetyl-CoA to 3-ketohexanoyl-CoA as required by the claims, citing Schubert, Haywood, et al., *FEMS Microbiol. Lett.* 52:91-96 (1988) ("Haywood 1), Haywood, et al., FEMS Microbiol. Lett. 52:259-64 (1988) ("Haywood 2"), Peoples and the present specification. The Examiner's attention is respectfully drawn to the claim limitations. The claims require not only that the organism express the requisite thiolase, but also that the enzymes be expressed in a sufficient amount to produce polyhydroxybutyrate-co-3-hydroxyhexanoate from butanol or butyrate as the carbon source. The Examiner cited Schubert as evidence that *R. eutropha* comprises an endogenous 3-keto-thiolase gene (phbA). While this confirms that such an enzyme was known, it does not disclose that those skilled in the art had combined it with the other necessary enzymes to produce a copolymer of a short chain substrate, butyrate, with a long chain substrate, hexanoate, in a single organism, provided only with a short chain substrate, butyrate or butanol.

A conclusion cannot be drawn using the disclosure in Schubert, that *R. eutropha* employed in Fukui for the production of polyhydroxybutyrate-co-3-hydroxyhexanoate inherently contains a 3-ketothiolase gene which encodes an enzyme that can convert butyryl-CoA and acetyl-CoA to 3-keto-hexanoyl-CoA as required by the claimed method. There is no mention in Schubert of any enzyme in *R. eutropha,* catalyzing the formation of 3-keto-hexanoyl-CoA from butyryl-CoA and acetyl-CoA. Many thiolases are expressed in different bacteria. The specification at least at page 11, lines 19-30 discloses the thiolases present in *R. eutropha*, indicating that while enzyme A is strictly specific for acetoacetyl-CoA and 3-ketovaleryl CoA, enzyme B has 1-2% activity for the higher 3-ketoacyl CoA's. The specification further states that synthesis of 3-hydroxyhexanoyl-CoA monomers with the PHB enzymes from *R. eutropha* can be improved by identifying and using thiolases and/or reductases with advantageous specificity for 3-ketohexanoyl-CoA. This is confirmed by the results demonstrated in Table 2 of Haywood 1 which shows that enzyme B has a much lower specificity for longer higher 3-ketoacyl CoA's (See page 94). The Examiner cited to Haywood 1 on page 94, which states that the Km for acetoacetyl-CoA was much higher for enzyme B than that for 3-ketoocatanoyl-CoA, indicating that the enzyme would be active at very low concentrations of longer-chain substrates. The examiner alleges this would be recognized by one of ordinary skill in the art as levels of substrate that reflect "physiological concentrations" found in the cell. This makes no sense. One is not dealing with physiological concentrations. This organism was developed for a high yield, inexpensive, commercial process. One needs to provide large amounts of appropriate substrate, not "physiological concentrations", whatever that might mean.

The Examiner also asserted that Peoples at column 21, lines 10-21 points to using the thiolase gene from *R. eutropha* because it condenses butyryl CoA and acetyl CoA. Peoples

discloses that the thiolase from *R. eutropha* has a broad substrate specificity, whichis demonstrated in Haywood 1. However, the issue here is whether the thiolases in *R. eutropha* can enable a genetically engineered organism to use butyrate or butanol for the production of a PHA polymer with 3-hydroyhexanoate units and 3-hydroxybutyrate units from butanol or butyrate by virtue of having the ability to convert butyryl-CoA and acetyl-CoA to 3-kethexanoyl-CoA in a sufficient amount to produce the co-polymer. Appellants maintain that the Examiner has provided no evidence demonstrating that the thiolases from *R. eutropha* can confer this ability to a genetically modified organism.

The Examiner also alleged that in none of Appellants' examples do they teach or practice utilization of a 3-ketothiolase that condenses butyryl-CoA and acetyl-CoA. The Examiner is respectfully reminded that a rejection of the claims as anticipated by a prior art reference should be limited to analysis of whether the prior art reference in question recites all the elements of the claim, which Appellants have proven Fukui does not. Further, the Examiner's attention is drawn to the specification at least from page 26, line 24 until page 27, line 2. Even if the Examiner's assertions were correct, Fukui does not disclose a method for PHA production that involves using cheap feedstock such as butyrate or butanol. From the discussion above, it is clear that Fukui does not disclose the claimed method.

Claims 1, 7, and 14

Claims 1 and 7 define a method for the biological production of PHA containing 3-hydroxybutyrate and 3-hydroxyhexanoate that requires bacteria engineered with enzyme(s) to generate 3-hydroxybutyrate and 3-ketohexanoyl-CoA from butyryl-CoA and acetyl-CoA. The method provides genetically engineered bacteria expressing a 3-ketothiolase gene which encodes an enzyme capable of converting butyryl-CoA and acetyl-CoA to 3-ketohexanoyl-CoA. The

bacteria also encode an acetoacetyl CoA reductase gene that encodes an enzyme that converts the 3-ketohexanoyl-CoA to 3-hydroxyhexanoyl-CoA, and a polyhydroxyalkanoate polymerase that polymerizes 3-hydroxybutyryl-CoA and 3-hydroxyhexanoyl-CoA. The method ensures that the enzymes are expressed in sufficient amounts to produce polyhydroxybutyrate-co-3-hydroxyhexanoate, and provides substrates sequentially utilized by each enzyme in the pathway, as well as grows the bacteria under conditions wherein polyhydroxybutyrate-co-3-hydroxyhexanoate is produced.

Fukui appears to disclose providing a thiolase, reductase and polymerase. Fukui does not provide a method that utilizes butanol or butyrate to produce a copolymer as claimed, for the reasons discussed above.

Claim 14 is dependent on claim 1 and requires that the bacteria be selected from the group consisting of *E. coli, Klebsiella, Ralstonia, Alcaligenes, Pseudomonas,* and *Azotobacter*.

Claim 16

Claim 16 is dependent on claim 1 and requires that the bacteria express a gene encoding D-specific enoyl-CoA hydratase. There is no disclosure in Fukui of such an enzyme being present.

In summary, claims 1, 7 and 16 are novel over Fukui.

Claims 10, 18-21 and 36

Claim 18 is dependent on claim 1, and requires the bacteria express one or more fatty acid biosynthetic genes. Claim 19 is dependent on claim 18, and require the fatty acid biosynthetic enzymes convert 3-hydroxyacyl-ACP to 3-hydroacyl-CoA. Claim 10 is dependent on claim 19, and requires that the bacteria further comprise a gene encoding 3-hydroxyacyl-ACP-coenzyme A transferase. Claim 20 is dependent on claim 19 and requires that enzymes are

selected from the group consisting of 3-hydroxyacyl-ACP-coenzyme-A transferase, acyl-ACP thioesterase, and acyl-CoA synthase. Claim 21 is dependent on claim 20 and requires that the enzymes are acyl ACP thioesterase and acyl-CoA synthase. Claims 36 and 38 depend from claim 18 and further specify the enzymes.

As discussed above, Fukui does not disclose a method of production of PHA containing 3-hydroxybutyrate and 3-hydroxyhexanoate by providing genetically engineered bacteria capable of producing both hydroxybutyrate and hydroxyhexanoate from butyryl-CoA and acetyl CoA nor bacteria that can produce PHA containing 3-hydroxybutyrate and 3-hydroxyhexanoate from short chain carbons such as butyric acid or butanol. Accordingly, the methods of claims 10, 18-21 and 35-39 are novel.

(e) Rejections Under 35 U.S.C. § 103

The Legal Standard

When applying 35 U.S.C. § 103, the following tenets of patent law must be adhered to:

(a) determining the scope and contents of the prior art;

(b) ascertaining the differences between the prior art and the claims in issue;

(c) resolving the level of ordinary skill in the pertinent art; and

(d) evaluating evidence of secondary consideration.

Graham v. John Deere, 383 US 1, 17-18, 148 U.S.P.Q. 459, 467 (1966). These four factors are traditionally referred to as the Graham factors.

The Graham factors were recently affirmed by the U.S. Supreme Court in *KSR International Co. v. Teleflex, Inc.*, 127 S. Ct. 1727, 82 U.S.P.Q.2d 1385 (2007). In its analysis of the obviousness standard, the Court did not totally reject the Federal Circuit's prior use of "teaching, suggestion, or motivation" as a factor in the obviousness analysis. Rather, the Court

recognized that a showing of "teaching, suggestion, or motivation" to combine the prior art to meet the claimed subject matter may provide a helpful insight in determining whether the claimed subject matter is obvious under 35 U.S.C. § 103(a).

The Court also warned against the use of hindsight analysis in making an obviousness determination. The Court stated, "A factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon *ex post* reasoning." (*KSR*, 127 S. Ct. at 1742, citing *Graham*, 383 U.S. at 36 (warning against a "temptation to read into the prior art the teachings of the invention in issue" and instructing courts to "'guard against slipping into the use of hindsight'" (quoting M*onroe Auto Equipment Co. v. Heckethorn Mfg. & Supply Co.*, 332 F.2d 406, 412, 141 U.S.P.Q. 549 (6th Cir. 1964))).

In response to the *KSR* decision, the Deputy Commissioner for the USPTO issued a memorandum stating: "[I]n formulating a rejection under 35 U.S.C. § 103(a) based upon a combination of prior art elements, it remains necessary to identify the reason why a person of ordinary skill in the art would have combined the prior art elements in the manner claimed." Memorandum from Margaret A. Forcarino to Technology Center Directors (May 3, 2007).

Analysis

(i) Claims 1, 6, 7, 10, 14, 16-21, and 35-39 are non-obvious over Fukui in view Mascarenhas and further in view of Schubert.

Mascarenhas

Mascarenhas discloses methods and compositions for inserting a copy of a heterologous gene into the chromosome of a host cell such as *E. coli*, through the use of a chromosomal transfer DNA, a circular DNA, non-self-replicating DNA carrying a site-specific recombination site.

Schubert

Schubert discloses the genes in *A eutrophus* which are involved in the synthesis of PHB. The heterologous expression of the *A. eutrophus* PHB synthase genes in *E. coli* and the formation of PHB granules in recombinant strains of E. coli provided some evidence that all three genes of the PHB-synthetic pathway are clustered in *A. eutrophus* (Schubert, abstract).

Boynton

Boynton discloses the cloning, sequencing and expression of clustered genes encoding β-butyryl-coenzyme A, crotonase and butyryl-coenzyme A dehydrogenase and related enzymes from *C. acetobutylicum*.

Voelker

Voelker discloses plant thioesterases, means to identify such proteins, amino acids and nucleic acids sequences associated with such proteins, and methods to obtain and/or use plant thioesterases.

Fukui, Mascarenhas, Schubert, Boynton and Voelker in Combination

Claims 1, 6-7 and 16

As disclosed above in response to the 102 rejection, Fukui does not disclose all the elements of the claims. None of the secondary references make up for this deficiency. The claimed method provides the ability to produce PHA containing 3-hydroxyhexanoate and 3-hydroxybutyrate from cheap carbon sources such as butyrate or butanol in genetically engineered organisms. This is one important aspect of this application, which is neither disclosed nor suggested in the prior art. In order for the organism of choice to be able to use butyrate or butyric acid to produce PHA containing 3-hydroxybutyrate and 3-hydroxyhexanoate, the organism has to express a thiolase that can condense 3-hydroxybutyryl CoA and acetyl CoA in a sufficient amount to produce PHA containing 3-hydroxybutyrate and 3-hydroxyhexanoate. For reasons discussed above, Fukui does not disclose the production of PHA containing 3-hydroxybutyrate and 3-hydroxyhexanoate in a genetically engineered organism that uses butyrate or butanol.

Fukui does not disclose bacteria encoding genes that enable the production of the 3-hydroxyhexanoate co-monomer from butyryl-CoA and acetyl CoA in sufficient amount to accumulate PHA containing hydroxyhexanoate units and hydroxybutyrate units. Neither Mascarenhas, Schubert nor Voelker make up for this deficiency. The Examiner depended on Mascarenhas for providing the advantages of integration of the gene into the bacterial chromosome. The Examiner has provided no reasoning why one of ordinary skill in the art would combine Fukui with Schubert, Boynton and Voelker. However, a combination of the prior art as the Examiner has done does not yield all the elements of the claims, nor provide a reasonable expectation of success.

The Examiner depended on Haywood 1 for alleging that *A. eutrophus* (now *R. eutropha*) inherently expresses a thiolase that would condense 3-hydroxybutyryl CoA and acetyl CoA; thus, the disclosure by Fukui, of production of PHA with 3-hydroxyheaxanoate units in mutant *A. eutrophus* expressing only the PHAC gene from *A. caviae* recites all the elements of the claims. As stated in Fukui and cited by the Examiner "apparently, *A. eutrophus* cells have the ability to supply (R)-3-hydroxyhexanoyl-CoA thioester intermediate from these carboxylic acids" The Examiner's attention is drawn to the fact that the carboxylic acids Fukui is referring to are hexanoate and octanoate. A conclusion cannot be drawn from this disclosure in Fukui that *A. eutrophus* has the ability to provide sufficient 3-hydroxyhexanoate intermediates and 3-hydroxybutyrate from butyrate, since having the ability to provide such intermediates from octanoate and hexanoate in sufficient amount for the accumulation of PHBH does not necessarily confer to the bacteria the ability to generate the intermediate from butyrate; different enzyme systems are involved (Compare Fig 4 in Fukui and Figure 3 of the present application). Moreover, given the dual pathway, and the fact that the naturally occurring organisms would produce poly(3-hydroxybutyrate), not a copolymer, there is no expectation that one would obtain the copolymer by feeding the organism butanol or butyrate, rather than the homopolymer.

Fukui (Fig 4) shows a proposed pathway for the production of 3-hydroxyhexanoyl-CoA intermediate (Fukui, Figure 4). It is clear that this step does not involve a thiolase. Nowhere in Schubert is it disclosed that the thiolase from *A. eutrophus* is capable of catalyzing the condensation of butyryl-CoA and acetyl-CoA. Thus, a skilled artisan would have no motivation to combine these references to define a method for PHA production from butyryl-CoA and acetyl-CoA, with any expectation of success.

With respect to utilization of butyrate recited in claim 1, the Examiner cited to Schubert and Doi as disclosing the utilization of butyrate by *R. eutropha*. However, such utilization leads to the production of polymers with hydroxybutyrate and hydroxyvalerate units. The issue is not only whether the bacteria can utilize butyrate, the claimed method also requires that the bacteria will produce a copolymer containing 3-hydroxyhexanoate and 3-hydroxybutyrate from a single short chain carbon source, either butanol or butyrate. Absent some modification to the known bacteria that would change production to the claimed copolymer which still utilized the same substrate, this would not occur. Such a modification is neither disclosed nor suggested in Schubert or Doi. The Examiner has provided no reason why one of ordinary skill in the art would combine Fukui with Schubert, Doi, Boynton or Voelker. However, even if one did, one would not arrive at the claimed method for reasons set forth above.

Therefore, claims 1, 6-7 and 16 are not obvious over Fukui, in view of Mascarenhas and further in view of Schubert, Boynton or Voelker.

Claims 10, 17-21 and 35-39

As discussed above, none of the cited prior art disclose a method of making PHA containing 3-hydroxybutyrate and 3-hydroxyhexanoate using butyryl-CoA and acetyl-CoA as intermediates, nor a thiolase that can catalyze condensation of these substrates in a pathway to produce PHA.

Mascarenhas merely discloses the expression of heterologous DNA in a host cell. The Examiner has provided no reasoning why one of ordinary skill in the art will be motivated to combine Mascarenhas with Fukui with respect to claims 10, 18-21 and 35-39.

The Examiner depended on Boynton for disclosing the three enzymes recited in claim 17 and on Voelker for disclosing an ACP-thioesterase. According to the Examiner, one of ordinary

skill in the art would have been motivated by the fact that including an acyl ACP-thioesterase or any combination of enzymes that convert 3-hydroxy-ACP to 3-hydroxyl-CoA (claims 10 and 18-21) would provide substrate for the PHA polymerase taught by the method of Fukui without the requirement of adding hexanoate or octanoate, thereby improving the efficiency of the process by Fukui.

Appellants are not clear how a motivation to avoid the provision of octanoate or hexanoate as carbon sources is a relevant motivation for a killed artisan to combine the references as the Examiner has done, for the purpose of arriving at the claimed method. It is also not clear how avoiding the addition of hexanoate or octanoate would make the process in Fukui more efficient. Even if it did, the claims do not require avoiding octanoate and hexanoate. The claims require the use of cheap carbon sources such as butyrate and butanol to make PHA containing 3-hydroxybutyrate and 3-hydroxyhexanoate. Furthermore, the combination of Boynton and Fukui as the Examiner has done to avoid the use of hexanoate and octanoate as feed stock would change the principle of operation of the method disclosed in Fukui. Such a combination is not allowed (See MPEP §2143.01 (IV)). None of the cited art either alone or in combination makes up for the deficiencies in Fukui. Therefore, claims 10, 17-21 and 35-39 are not obvious over Fukui in view of Mascarenhas and further in view of Schubert, Boynton and Voelker.

(f) Summary and Conclusion

For a reference to anticipate under 102 (b), it must recite **each and every** claim limitation. Fukui does not meet this requirement. Fukui fails to explicitly disclose each claim limitation.

35 U.S.C. 103 is very clear: the prior art must disclose the claimed elements and the prior art must provide the motivation to combine as applicant has done, with a reasonable expectation of success. As the Supreme Court affirmed in KSR, one must look at each case on its facts. Appellants here have surprising results: they have shown that one can engineer a bacteria to utilize a short chain substrate, available cheaply and in quantity, butanol or butyrate, which bacteria would normally use to make the homopolymer poly(3-hydroxybutyrate), and instead make the more valuable copolymer, PHA containing 3-hydroxybutyrate and 3-hydroxyhexanoate.

Appellants are the first to show genes from two separate pathways, those involved in production of substrate from short carbon chain sources as well as those involved in production of a polymer, into an organism and demonstrate a successful outcome. None of the cited art leads one of skill in the art to utilize genes from two pathways, substrate formation and polymer production, as appellants' claim.

The Examiner has cited no art disclosing a method of making polyhydroxybutyrate-co-3-hydroxyhexanoate by providing genetically engineered bacteria expressing a 3-ketothiolase gene that converts butyryl-CoA and acetyl-CoA to 3-ketohexanoyl-CoA, using cheap feed stock such as butanol and butyrate.

Therefore, the cited art cannot make obvious the claimed method.

For the foregoing reasons, Appellants submit that claims 1, 6, 7, 10, 14, 16-21, and 35-39 are novel and non-obvious.

Respectfully submitted,

/ Patrea L. Pabst /

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Date: June 29, 2007

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